# Single Arm Phase II Study of Myeloablative Allogeneic Hematopoietic Stem Cell Transplantation for Acute Lymphoblastic Leukemia (ALL) in Older Patients Using Fludarabine and Total Body Irradiation (FluTBI) Regimen

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**Bone Marrow Transplantation and Cellular Therapy Program**

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# 1 Background

## 1.1 Allogeneic Hematopoietic Stem Cell Transplantation (allo HCT) for Acute Lymphoblastic Leukemia (ALL)

Although acute lymphoblastic leukemia (ALL) in children has become curable in 80% of cases,[1] the treatment outcome of adult ALL have been poor with cure rate of 30-40%.[2] This discrepancy may be explained, at least partially, by the difference in cytogenetic abnormality profile between children and adult ALL.[3] In Adult ALL, the complete remission rate is 70-90% with upfront therapy, but relapse rate thereafter is high. Thus long term survival can only be achieved in 30-40% of patients, and around 10% in patients above the age 60.[3, 4] In order to improve outcome of adult ALL, allo HCT has been used as a consolidation for patients in the 1st complete remission (CR).[5-7] Advanced age and limited performance status are associated with increased non-relapse mortality (NRM). Data from the European Group for Blood and Marrow transplantation (EBMT) showed NRM rate of about 30% in adult ALL after allo HCT.[8]

Results of the international trial of adult ALL (MRC UKALL XII/ECOG E2993) showed superior outcome for allo HCT in adult patients with standard-risk ALL in CR1 when compared with autologous HCT. The study enrolled ALL patients between 15 and 49 year old (the upper limit has been changed to 54 years) and proceed to allogeneic HCT if HLA matched sibling donor is available. Conditioning regimen was total body irradiation (TBI) 1320 cGy＋Etoposide 60 mg/kg. Patients in remission with no matched sibling were randomized to either chemotherapy maintenance or autologous HCT. They reported that relapse rate was significantly lower in cases with a donor compared to the ones without a donor in the analysis on Philadelphia chromosome negative (Ph-) cases, thus supported the presence of graft-versus leukemia (GVL) effect. In the standard risk cohort, patients with a donor showed better survival (62% vs. 52%, P = 0.02). In the high-risk cohort (age older than 35, or high WBC count at the time of diagnosis – more than 100,000/μl in B-lineage, more than 30,000/μl in T-lineage), however, survival was not significantly different as NRM was high in the transplanted patients (41% vs. 35%, P = 0.2).[9] In this study, the NRM rate was 20% and 39% in the young (< 35 years) and older patients underwent HCT, respectively.[9] It was suggested from this result that advantage of transplant may be more apparent if NRM can be decreased in older age group by using less toxic conditioning regimens.

### 1.2 Conditioning regimens for allo HCT in ALL

The conditioning regimen being used most often for ALL has been the combination of cyclophosphamide (Cy) and total body irradiation (TBI) called CyTBI. The BuCy regimen, which replaced TBI of CyTBI with busulfan (Bu) has been shown to have comparable outcome to CyTBI in acute myelogenous leukemia (AML)[10], but inferior outcome in pediatric ALL.[11] The combination of TBI and etoposide (Etp) has been associated with less relapse rate than CyTBI in second CR.[12] In addition, a randomized trial for pediatric ALL showed that TBICyEtp was superior to BuCyEtp in unrelated donor transplant with 3-year event-free survival (EFS) of 57% and 20% for both groups respectively (P=0.04). This randomized prospective study suggests that TBI has more favorable outcome compared to Bu in pediatric patients with ALL undergoing allo HCT. The City of Hope (COH) group has also reported EtpTBI regimen (TBI dose of 1320 cGy) in adult ALL.[13] These reports provide evidences for efficacy of TBI-based containing regimens in adult ALL.

### 1.3 Reduced-intensity allo HCT in ALL

In reduced-intensity transplant (RIT), less intense conditioning is used with less regimen-related toxicity, but anti-tumor effect is more dependent on GVL effect, as the anti-tumor activity of conditioning regimen itself is reduced. GVL may be less effective in ALL compared to myeloid tumors, such as AML and myelodysplastic syndrome (MDS).[14, 15] For this reason, recurrence of disease may be more of a concern. There is paucity of data of RIT in ALL. Arnold et al reported 22 cases of RIT for ALL using FluBu2 (fludarabine/busulfan) with or without ATG (anti-thymoglobulin). Among the cases who received RIT as the first transplant (11 cases) or second transplant (11 cases), 3 and 1 cases survived for a long time, respectively. All these 4 cases were transplanted at remission.[16] Martino et al reported 27 cases who received RIT for ALL.[17] A total of 23 out of 27 cases received a conditioning regimens based on Flu (90-150 mg/m2)-Melphalan (Mel) (140mg/m2). Two-year overall survival (OS) was 31%, and cumulative incidence of progression was 70% in cases without GVHD, 35% in cases with GVHD, thus suggesting the presence of GVL effect in ALL. Only 5 cases relapsed out of 15 cases who received RIT at CR.[17] A multi-center retrospective analysis of 33 cases of RIT for ALL in Japan was published by Hamaki et al.[18] Conditioning regimens were either FluBu2 or FluMel in most of the cases. The disease status at transplant was CR1 (13), CR2 (6) or non-remission (14), and 14 cases of Ph+

ALL were included. The 2-year progression-free survival (PFS) and OS were 19% and 30%, respectively. PFS at 1-year was 31% in cases in CR1/CR2 at the time of transplant, and 29% in cases not in CR[18]. Another study reported data of 43 patients of ALL in CR2 who underwent RIT after conditioning regimen of Flu (90 mg/m2) Cy (1050 mg/m2) Bu (8 mg/kg).[19] Regimen-related toxicity was acceptable, but relapse rate was as high as 70%, and OS at 3 years post-transplant was 30%. Mohty et al published a retrospective analysis of 97 patients who underwent RIT for ALL from EBMT[20]. It is difficult to assess the conditioning regimens in this study as they used various regimens, but OS and relapse rate (RR) at 2 years were 52% and 40% in cases transplanted at CR1, 27% and 63% in cases who had the transplant beyond CR1, 20% and 49% in cases transplanted at non-remission. For non-remission cases, the NRM was as high as 44%. The COH group reported their preliminary data of promising results of FluMel conditioning (Flu 125mg/m2 and Mel 140mg/m2).[21] FluMel regimen has also been used to salvage patients who relapsed after initial allo HCT.[22] Higher dose of Flu of 160 mg in combination with Mel and thiotepa was shown to be feasible conditioning regimen for allo HCT.[23] Another study reported the outcome of 51 patients with ALL who were > 50 years old or younger with high risk disease. In this study, patients underwent allo HCT using conditioning regimen of Flu TBI 2 Gy.[24] The study showed 3-year overall survival of 34%. The 3-year relapse rate was 40% and the NRM was 28%. Thus the use of reduced intensity regimen for ALL has become out of favor for high relapse rate that forfeits the benefit of reduced NRM.

### 1.4 Conditioning regimen with FluTBI

Most commonly used myeloablative conditioning regimen for relatively young patients with hematologic malignancies are CyTBI or BuCy. High-dose Cy may result in or contribute to severe regimen-related toxicities such as cardiac toxicity, hemorrhagic cystitis, and sinusoidal obstruction syndrome (SOS) previously known as veno-occlusive disease (VOD) of liver. Fludarabine is less toxic and more immunosuppressive (thus facilitating engraftment) compared to high-dose Cy.[25] Based on these findings FluBu4 regimen has been developed by replacing Cy of BuCy with Flu. Busulfan dose in this regimen is myeloablative (4-day dosing), as opposed to 2-day regimen of Bu in RIT regimen, FluBu2.[26] This regimen was reported to be safe and well tolerated than full intensity myeloablative regimens. The use of myeloablative regimen of FluTBI (8 Gy) was shown to result in successful engraftment in patients with AML with acceptable NRM rate.[27] However,

the addition of melphalan to FluTBI regimen resulted in significantly high NRM of 40% in a retrospective data from Japan.[28]

Since the use of TBI-based conditioning was shown to be more appropriate for ALL, a combination of Flu TBI may be more effective and better tolerated than Flu Mel or Flu Bu regimens for ALL. We will investigate the safety and efficacy of a myeloablative regimen of Flu TBI 12 Gy regimen for ALL patients who are not eligible for Cy TBI conditioning regimen.

# 2 Objectives and Endpoints

## 2.1 Primary Objective:

To establish the efficacy of allo HCT in older ALL patients using myeloablative FluTBI conditioning regimen.

## 2.2 Secondary Objectives:

To assess the safety and toxicity of allo HCT in older ALL patients using myeloablative FluTBI conditioning regimen.

## 2.3 Primary Endpoints

The primary study endpoint is disease-free survival (DFS) at one year post-transplant.

## 2.4 Secondary Endpoints

- One-year overall survival.
- Regimen related toxicity within the first 100 days post-transplant.
- Time to neutrophil and platelet engraftment. Neutrophil engraftment is defined as the first of 3 consecutive days with an absolute neutrophil count (ANC) > 500/μL. Platelet engraftment is defined as the first of 3 consecutive days with a platelet count > 20,000/μL without platelet transfusion for 7 days.
- Incidence and severity of acute and chronic GVHD.
- Immune Reconstitution over time.
- Relapse rate at 2 years post-transplant.

# 3 Patients Eligibility Criteria

## 3.1 Inclusion Criteria

1) Disease Criteria:
    - ALL in complete remission (CR) at the time of transplant. Remission is defined as "less than 5.0% bone marrow lymphoblasts by *morphology*," as determined by a bone marrow aspirate obtained within 2 weeks of study registration.
    - Philadelphia chromosome positive ALL is allowed.
    - Lymphoid blastic crisis of CML will be included (provided that patients achieve CR).
2) Age Criteria: Equal or above age 40 and up to 65 years. If younger than 40, there must be comorbidities which preclude the patient to undergo CyTBI conditioning regimen.
3) Organ Function Criteria: All organ function testing should be done within 35 days of study registration.
4) Cardiac: Left ventricular ejection fraction (LVEF) ≥ 50% by MUGA (Multi Gated Acquisition) scan or echocardiogram.
5) Pulmonary: FEV1 (Forced expiratory volume in 1 second) and FVC (Forced vital capacity) ≥ 50% predicted, DLCO (alveolar diffusion capacity for carbon monoxide) (corrected for hemoglobin) ≥ 50% of predicted.
6) Renal: The estimated creatinine clearance (CrCl) must be equal or greater than 60 mL/min/1.73 $m^2$ as calculated by the Cockcroft-Gault Formula:
CrCl = (140-age) x weight (kg) x 0.85 (if female)/72 x serum creatinine (mg/dL).
7) Hepatic:
    - Serum bilirubin ≤2.0 g/dL
    - Aspartate transaminase (AST)/alanine transaminase (ALT) ≤2.5 × ULN
    - Alkaline phosphatase ≤ 2.5 × ULN
8) Performance status: Karnofsky ≥ 70%
9) Consent: Patient must be informed of the investigational nature of this study in accordance with institutional and federal guidelines and have the ability to provide written informed consent prior to initiation of any study-related procedures, and ability, in the opinion of the principal investigator, to comply with all the requirements of the study.

10) Presence of a willing adult HLA-matched sibling (excluding identical twin) or HLA-matched unrelated donor meeting all the criteria for routine allo HSCT. All donors will be evaluated for eligibility and suitability per the standard of care according to the FACT and NMDP guidelines.

### 3.2 Exclusion Criteria

1) Non-compliant to medications.
2) No appropriate caregivers identified.
3) HIV1 (Human Immunodeficiency Virus-1) or HIV2 positive
4) Active life-threatening cancer requiring treatment other than ALL
5) Uncontrolled medical or psychiatric disorders.
6) Uncontrolled infections, defined as positive blood cultures within 72 hours of study entry, or evidence of progressive infection by imaging studies such as chest CT scan within 14 days of registration.
7) Active central nervous system (CNS) leukemia
8) Preceding allogeneic HSCT
9) Receiving intensive chemotherapy within 21 days of registration. Maintenance type of chemotherapy will be allowed.

## 4 Study Treatment

### 4.1 Conditioning regimen

The transplantation conditioning regimen will be fludarabine and TBI (1200 cGy) as follows:

Day -7 Fludarabine 40 mg/$m^2$ IV (Intravenous),

Day -6 Fludarabine 40 mg/$m^2$ IV

Day -5 Fludarabine 40 mg/$m^2$ IV

Day -4 Fludarabine 40 mg/$m^2$ IV

Day -3 TBI 2 Gy x 2

Day -2 TBI 2 Gy x 2

Day -1 TBI 2 Gy x 2,

Day 0 Transplant

**Fludarabine**

- Fludarabine dose (40 mg/$m^2$/day) will be based on adjusted ideal body weight if the actual body weight is > ideal body weight.
    - Adjusted ideal body weight = ideal weight + (actual Weight-ideal weight) x 0.4.
    - If actual weight is lower than ideal weight, we will use actual weight.
- Fludarabine will be given by 30 minutes IV infusion on days -7 to -4.

**Total Body Irradiation (TBI)**

- TBI is given at the total dose of 12 Gy, divided in 6 fractions over 3 days between day -3 and day -1.
- 10 mg of dexamethasone IV will be given daily during TBI.

### 4.2 Allogeneic Stem Cell Collection and Infusion

Stem Cell Source

- The source of donor stem cells will be peripheral blood stem cells (PBSC) or bone marrow (BM). Cord blood will not be allowed.

Stem Cell Mobilization/Collection

- PBSC mobilization and collection procedures and BM collection procedures will follow the institutional practice.

Stem cell infusion:

- PBSC from a related donor should be infused into the patient on the same day of collection. However, given the logistics of unrelated donor collections, unrelated PBSC may be infused within 48h of collection.
    - For PBSC: The recommended stem cells dose is 3.5-10 x $10^6$ CD34 cells/kg recipient weight.
    - For marrow infusions: The recommended cell dose is $\geq$ 2.0 x $10^8$ mononuclear cells/kg recipient weight.
    - Note: The day of the stem cell/marrow infusion will be defined as day 0. If more than one day of infusion is required, then these days are defined as day 0a, day 0b accordingly. The first day after the last stem cell infusion will be defined as day 1.

### 4.3 Maintenance treatment after transplant

Patients with Philadelphia positive ALL, will receive tyrosine kinase inhibitors after transplant per NCCN guidelines.[29]

Patients may also receive post transplant intrathecal antimetabolite therapy using cytarabine based on disease characteristics and the patient's history of CNS prophylaxis at the discretion of the treating physician and standard practice.

### 4.4 GVHD prophylaxis

- GVHD prophylaxis will be tacrolimus (0.03 mg/kg/day beginning on day -3) and methotrexate (5 mg/m$^2$ on days 1, 3, 6, and 11). Patients will be converted to oral tacrolimus when tolerated. Tacrolimus will be continued until day +100 and then tapered if there is no evidence of active GVHD. Tacrolimus serum level will be followed up per institutional practice.
- Leucovorin 5 mg/m$^2$ IV every 6 hours x4 doses on days 2, 4, 7, and 12 is optional if severe mucositis.
- Matched unrelated donor recipients will receive, in addition to above, rabbit anti-thymocyte globulin (rATG) IV at 1.5 mg/kg on days -7, -6, and -5 for a total rATG dose of 4.5mg/kg. If unable to receive rATG the use of horse derived ATG is allowed per physician discretion.

- The use of Mycophenolate Mofetil (MMF) and other appropriate immunosuppressive agents are allowed in high risk patients at the discretion of the transplant physician.

### 4.5 Infection Prophylaxis

Antibacterial, antiviral and antifungal coverage during neutropenia until engraftment will be at the discretion of the transplant physician's clinical practice standards.

The following is a suggested prophylaxis and monitoring regimen:

#### 4.5.1 Recommended Fungal Prophylaxis

Patients will receive Voriconazole 200 mg PO BID until day +75 post-transplant. For patients who develop elevated liver enzymes (AST or ALT > 2.5 x ULN or total bilirubin > 2.5 x ULN), micafungin 50mg IV/day may replace voriconazole until AST, ALT and/or total bilirubin decline to grade I level (<2.5x ULN).

### 4.5.2 HSV / VZV Prophylaxis

Herpes simplex virus / Herpes zoster virus (HSV/VZV) infection prophylaxis will be done using Acyclovir or valacyclovir daily until day +365 post-transplant.

### 4.5.3 CMV (Cytomegalovirus) preemptive therapy

In all cases in which either the patient or donor are seropositive for CMV pre-transplant, CMV-antigenemia or quantitative PCR testing will be obtained from blood weekly, starting from the time of neutrophil engraftment until at least day 100. Patients whose CMV PCR or antigenemia assays become positive post-transplant shall be treated per the transplant physician's clinical practice standard.

### 4.5.4 PCP (Pneumocystic pneumonia) prophylaxis

PCP prophylaxis should be initiated approximately day +30 post-transplant, provided the patient has met the engraftment criteria for neutrophils and platelets. Primary PCP prophylaxis shall be trimethoprim-sulfamethoxazole (TMP-SMX) single strength daily for 5 days a week or double strength (Bactrim DS) PO once a day for 2 or 3 days a week (preferred PCP prophylaxis). If intolerant to TMP-SMX, pentamidine 300 mg inhaler (or IV) every 4 weeks, dapsone 100 mg once a day or atovaquone (Mepron) 1500 mg PO daily may be used.

### 4.5.5 PCR testing

PCR testing for HHV6 and Adenovirus will be sent weekly starting approximately Day +20 until day 100.

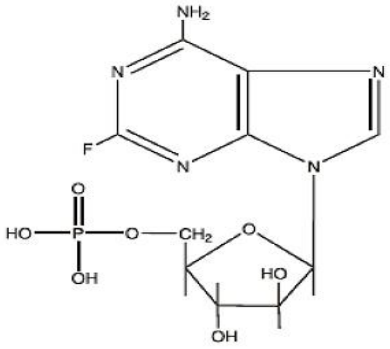
PCR testing for EBV will be sent every 2 weeks starting approximately Day +20 until day 100.

## 5 Drug Information

### 5.1 Fludarabine

5.1.1. Chemical Characteristics

Fludarabine phosphate (fludarabine) is an antimetabolite with chemical name 9H-Purin-6-amine, 2-fluoro-9-(5-0-phosphono- 0-D-arabino-furanosyl) (2-fluoro-ara-AMP). The molecular formula is $C_{10}H_{13}FN_5O_7P$ with molecular weight of 365.2.



5.1.2. Available Forms

IV Fludarabine is packaged as a white lyophilized solid cake in a vial. Each vial has 50mg fludarabine phosphate and only one vial is enclosed per carton.

5.1.3. Storage and Handling

Unopened vials of fludarabine should be stored at 20° to 25°C (68° TO 77°F); excursions permitted between 15° to 30°C (59° TO 86°F).

5.1.4. Toxicity

Toxicities reported as more than 10% incidence are:

Myelosuppression (neutropenia, thrombocytopenia, and anemia), fever and chills, infections, nausea and vomiting, pain, weakness, cough, pneumonia, dyspnea, diarrhea, anorexia, rash, edema.

Toxicities with expected incidences between 1% and 10% are:

Malaise, stomatitis, myalgia, paresthesia, visual disturbance, gastrointestinal bleeding, upper respiratory infection, diaphoresis, dysuria, urinary infection, sinusitis, hearing loss, hyperglycemia, headache, pharyngitis, hemoptysis, esophagitis, mucositis, hematuria, osteoporosis, alopecia, anaphylaxis, hemorrhage, dehydration, sleep disorder, depression, cerebellar syndrome, impaired mentation, allergic pneumonitis, epitaxis, bronchitis, hypoxia, liver failure, abnormal liver function, cholelithiasis, ARDS, respiratory distress, pulmonary hemorrhage, pulmonary fibrosis, respiratory failure, constipation, dysphagia, pruritus, seborrhea, renal failure, abnormal, renal function test, protenuria, hesitancy, angina, congestive, heart failure, arrhythmia, supraventricular

tachycardia, myocardial infarction, deep venous thrombosis, phlebitis, transient ischemic attack, aneurysm, cerebrovascular accident, arthralgia, tumor lysis syndrome.

5.1.5. Administration

IV fludarabine is prepared by adding sterile water to the white solid cake. Reconstituted in 2mL of sterile water, the solid cake produces a solution with approximate concentration of 25mg/mL fludarabine phosphate. Follow the institutional guidelines for further preparation and administration procedures of fludarabine.

Reconstituted IV fludarabine contains no antimicrobial preservative hence should be utilized within 8 hours of reconstitution. It should NOT be infused concomitantly with another intravenous solution of unknown compatibility.

## 5.2 Total Body Irradiation (TBI)

TBI will be administered per standard of care procedure as implemented by radiation oncologists. TBI alone for post-pubescent patients with dose/fractionation not exceeding 2 Gy x 6 is well within the tolerance of most normal organs for < 5% risk of severe late toxicity (organ failure or major dysfunction) by 5 years. Notable exceptions are risk of cataract development, bone marrow suppression, ovarian and testicular dysfunction. Also, there is a small risk of second malignancy. The most common acute effects include nausea, vomiting, diarrhea and painful swelling of the parotid glands.

When TBI is given in conjunction with other therapies in the transplant setting, there is additional risk of side effects including loss of appetite, dry mouth, difficult or painful swallowing , headache, stomatitis (sore throat/mouth), altered skin integrity, hair loss, swelling, increased risk for infection and/or bleeding, possible lung failure, dry cough, fatigue, anxiety, fever, possible liver failure, lung scarring, loss of vision, shortness of breath, sterility, heartburn, cystitis, sleep disturbances altered gastrointestinal and genitourinary function, neuropathy, fistulas, altered endocrine function, pericarditis and increased risk of a second cancer. Overall, the incidence of most major toxicity when radiation is given in conjunction with other therapy as outlined above is still low, rare, serious side effects are possible.

## 5.3 Tacrolimus

### 5.3.1. Chemical Characteristics

Tacrolimus is a macrolide immunosuppressant produced by Stretocyces Tsukubaensis. Tacrolimus has an empirical formulation of C44H69NO12-H2O and a formula weight of 822.05. Tacrolimus appears as white crystals or crystalline powder. It is practically insoluble in water, freely soluble in ethanol, and very soluble in methanol and chloroform. Tacrolimus inhibits T-lymphocyte activation, although the exact mechanism of action is not known. Experimental evidence suggests that tacrolimus binds to an intracellular protein, FKBP-12. A complex of tacrolimus-FKBP-12, calcium, calmodulin, and calcineurin is then formed and the phosphatase activity of calcineurin inhibited. This effect may prevent the dephosphorylation and translocation of nuclear factor of activated T-cells (NF-AT), a nuclear component thought to initiate gene transcription for the formation of lymphokines (such as interleukin-2, gamma interferon). The net result is the inhibition of T-lymphocyte activation (i.e., immunosuppression).

### 5.3.2. Available Forms

Tacrolimus is available for oral administration as capsules (tacrolimus capsules) containing the equivalent of 0.5 mg, 1mg and 5mg of anhydrous tacrolimus. Inactive ingredients include lactose, hydroxypropyl methylcellulose, croscarmellose sodium, and magnesium stearate. The 0.5 mg and 1 mg capsule shell contains gelatin and titanium dioxide, and the 5-mg capsule shell contains gelatine, titanium dioxide, and ferric oxide. Tacrolimus is also available as a sterile solution (tacrolimus injection) containing the equivalent of 5mg anhydrous tacrolimus in 1 ml for administration by IV infusion only. Each mL contains polyoxyl 60 hydrogenated castor oil (HCO-60), 200 mg, and dehydrated alcohol, USP, 80.0% v/v.

### 5.3.3. Storage and Handling

Tacrolimus capsules should be stored at room temperature between 15° and 30°C (59° and 86°F). Tacrolimus injection should be stored between 5° and 25°C (41° and 77°F).

### 5.3.4. Toxicity

Possible side effects of tacrolimus include: depressed kidney function, high blood sugar, high blood potassium, skin rash, headache, nausea, vomiting. Less common side effects are loss of appetite, sleep disturbances, vivid dreams, hallucinations, high blood pressure, seizure, decreased

level of consciousness, anemia, agitation, tremors, irritability, slurred speech, tingling in the hands and feet, pain in the palms and soles of the feet, weakness, and abnormal blood cell levels. All of these side effects are reversible by reducing the dose or discontinuing the drug. Rare fatal cases of severe allergic reactions have been reported in patients receiving cyclosporine and it is possible that similar reactions could also occur in patients receiving tacrolimus.

#### 5.3.5. Administration

Tacrolimus injection) must be diluted with NS or D5W before use. Tacrolimus is administered as a continuous infusion. Oral preparation will be administered on empty stomach every 12 hours.

### 5.4 Methotrexate

#### 5.4.1. Chemical Characteristics

Methotrexate is a folate antimetabolite that inhibits DNA synthesis. Methotrexate irreversibly binds to dihydrofolate reductase, inhibiting the formation of reduced folates, and thymidylate synthetase, resulting in inhibition of purine and thymidylic acid synthesis. Methotrexate is cell cycle specific for the S phase of the cycle. It is also used as the immunosuppressive agent, or to prevent/treat GVHD. The effect of this agent can be antagonized with the administration of leucovorin.

#### 5.4.2. Available Forms

Methotrexate Injection, USP is available in 50, 100, 200, and 250 mg single-dose vials of sterile, isotonic liquid containing no preservatives for parenteral administration.

Each 25 mg/mL, 2 mL (50 mg) vial contains methotrexate sodium equivalent to 50 mg methotrexate. Inactive ingredients are sodium chloride for isotonicity and sodium hydroxide and, if necessary, hydrochloric acid to adjust pH to approximately 8.5.

Each 25 mg/mL, 4 mL (100 mg) vial contains methotrexate sodium equivalent to 100 mg methotrexate. Inactive ingredients are sodium chloride for isotonicity and sodium hydroxide and, if necessary, hydrochloric acid to adjust pH to approximately 8.5.

Each 25 mg/mL, 8 mL (200 mg) vial contains methotrexate sodium equivalent to 200 mg methotrexate. Inactive ingredients are sodium chloride for isotonicity and sodium hydroxide and, if necessary, hydrochloric acid to adjust pH to approximately 8.5.
Each 25 mg/mL, 10 mL (250 mg) vial contains methotrexate sodium equivalent to 250 mg methotrexate. Inactive ingredients are sodium chloride for isotonicity and sodium hydroxide and, if necessary, hydrochloric acid to adjust pH to approximately 8.5.

Methotrexate sodium is also available in 20 mg vial of lyophilized powder. Methotrexate sodium for injection should be reconstituted with an appropriate sterile, preservative free medium such as NS or D5W. Reconstitute the 20 mg vial to a concentration no greater than 25 mg/mL. Reconstitute immediately prior to use.

5.4.3. Storage and Handling

Store at controlled room temperature, 20-25°C (68-77° F); excursions permitted to 15-30°C (59-86°F). Protect from light.

5.4.4. Toxicity

The most frequently reported adverse reactions associated with methotrexate use as GVHD prophylaxis include ulcerative stomatitis, leucopenia and suppressed hematopoiesis, nausea, and abdominal distress. Other frequently reported adverse effects are malaise, undue fatigue, chills, and fever, dizziness and decreased resistance to infection. Methotrexate may be associated with increased rates of pulmonary complications after transplantation. The risk of infections is due to the suppression of hematopoiesis after transplantation.

## 5.5 Thymoglobulin® (Anti-thymocyte Globulin [Rabbit]) (rATG)

5.5.1 Chemical Characteristics

Thymoglobulin® (Anti-thymocyte globulin [rabbit]) is a purified, pasteurized, gamma immune globulin obtained by immunization of rabbits with human thymocytes. Gamma immune globulin or Immunoglobulins are heavy plasma proteins, often with added sugar chains on N-terminal.

### 5.5.2 Available Forms

Thymoglobulin (Anti-thymocyte Globulin [Rabbit]) is available as sterile, lyophilized powder to be reconstituted with Sterile Water for Injections, EP.

Each package contains one 10 mL vial. The reconstituted preparation contains approximately 5 mg/mL of Thymoglobulin of which >95% is rabbit gamma immune globulin (IgG). The reconstituted solution has a pH of 7.0 ± 0.4. Human red blood cells are used in the manufacturing process to deplete cross-reactive antibodies to non T-cell antigens. The manufacturing process is validated to remove or inactivate potential exogenous viruses. All human red blood cells are from US registered or FDA licensed blood banks. A viral inactivation step (pasteurization, i.e., heat treatment of active ingredient at 60$_{o}$C/10 hours) is performed for each lot.

### 5.5.3 Storage and Handling

Store in refrigerator between +2°C and +8°C (36°F to 46°F). A higher temperature of ≤37°C during transport for a total excursion time of ≤10 days will do the product no harm. Protect from light. Do not freeze. Do not use after the expiration dated indicated on the label. Any unused drug remaining after infusion must be discarded.

### 5.5.4 Toxicity

Thymoglobulin adverse events are generally manageable or reversible. The most frequent reported adverse events (more than 25% of patients) include: fever, chills, leukopenia, pain, headache, abdominal pain, diarrhea, hypertension, nausea, thrombocytopenia, peripheral edema, dyspnea, asthenia, hyperkalemia, tachycardia, and infection.

Serious immune-mediated reactions have been reported with the use of Thymoglobulin and consist of anaphylaxis or severe cytokine release syndrome (CRS). Fatal anaphylaxis has been reported. Severe, acute infusion-associated reactions (IARs) are consistent with CRS and can cause serious cardiorespiratory events and/or death. IARs may occur as soon as the first or second infusion during a single course of Thymoglobulin treatment. During post-marketing surveillance, fever, rash, arthralgia and/or myalgia have been reported to occur 5 to 15 days after onset of Thymoglobulin therapy, indicating possible serum sickness. These symptoms are manageable with corticosteroid treatment. Infections, reactivation of infection, sepsis, malignancies including post-transplant lymphoproliferative disorder (PTLD) and other lymphomas as well as solid tumors have been reported after Thymoglobulin administration in combination with multiple immunosuppressive agents.

# 6 Required Observations

***Please see appendix B for a study calendar. Calendar needs updating to match***

## 6.1 Pre-Transplant (within 35 days prior to study registration / day of final transplant evaluation).

6.1.1 History and physical exam (include Karnofsky Performance Score within 14 days prior to registration)

6.1.2 Creatinine, AST, ALT, Alk Phos., and Total bilirubin

6.1.3 Echocardiogram or MUGA

6.1.4 Pulmonary function testing: FVC, FEV1, DLCO (corrected for hemoglobin).

6.1.5 Unilateral bone marrow aspirate and biopsy (within 35 days of registration), morphology and cytogenetics.

6.1.6 Cerebrospinal fluid examination is not required, unless clinically indicated.

## 6.2 Post-transplant required observations and follow-up plans

6.2.1. Bone marrow aspirate and biopsy specimen shall be collected for morphology examination and cytogenetics at day +30 (±7), +100 (±14), and 1 year (±45) post-transplant for all patients who are clinically stable and who have not demonstrated disease progression by that time point. In addition, a unilateral marrow aspirate will also be collected whenever a relapse is suspected.

6.2.2. Immune Reconstitution and Chimerism studies will be performed per BMT standard and/or as clinically indicated. The recommended timing of the labs is at Day +30 (±7), Day +60 (±7), Day +100 (±14), Day +180 (±21) and at 1 year (±45) post-transplant.

6.2.2. Patients need to be seen in clinic at least once a week until day 100 post-transplant and have acute GVHD assessment (using consensus criteria[30]) weekly (±3 days). Then they need to be seen at least once a month (±14 days) until 1 year after transplant to have chronic GVHD assessment.

6.2.3. Adverse Event and Toxicity monitoring will be performed at each visit date.

6.2.4. The patient will be followed at least for 2 years after transplant for survival and relapse. The follow up interval is determined as clinically necessary.

6.2.5. The patient who relapsed after transplant will be followed only for survival.

# 7 Statistical Considerations

## 7.1 Sample Size

This is a single center, single arm phase II study whose primary aim is to show an improvement in one-year DFS to 75% from an historical rate of ~ 45%. The estimated 1-year OS of ALL patients ≥ 20 years old undergoing allo HCT in first or subsequent CR is 55-65% according to the data of the Center for International Blood and Marrow Transplant Research (CIBMTR).[31] CIBMTR did not report DFS in this subset of adult patients. Patients with ALL who are ≥ 35 years old are known to have high-risk disease and lower survival.[3] A French study excluded patients who are ≥ 50 years from allo HCT and these patients (≥ 50 years) were randomized to either consolidation autologous HCT (auto HCT) or chemotherapy alone. The median DFS in this subset (≥ 50 years old) was 14 months and 3-year DFS was 24%.[32, 33] According to these data, we estimate that the 1-year DFS in patients ≥ 40 years old who receive allo HCT to be ~ 45%. We hypothesize that reduced toxicity myeloablative conditioning using FluTBI will improve this 1-year DFS to 75%. A maximum total of 16 evaluable patients will be enrolled to have a probability of .05 or less of concluding that the one year DFS rate exceeds 45% if it really is 45% and a probability of at least .80 of concluding that the one year DFS rate exceeds 45% if it is really .75%. We plan to enroll a total of 20 patients to allow for non-evaluable patients (patients who sign consent and do not get transplant).

We estimate enrolling 7-8 patients per year in the UAB transplant program.

## 7.2 Analysis Methods

Since all subjects will be followed for at least one year or until death, whichever is sooner, one year DFS can be thought of as a dichotomous (yes/no) variable. Thus a two stage design based on a binomially distributed random variable is appropriate. We have chosen a two stage design which will accrue 6 subjects in the first stage. If one or fewer achieve one year DFS the study will be terminated with the conclusion that the one year DFS rate does not exceed 45%. Otherwise, an additional 10 will be accrued for a total of 16. If 10 or fewer of these 16 achieve one year DFS we will conclude that the one year DFS rate does not exceed 75%. Otherwise we will conclude that it does exceed 45%. If the one year DFS rate is really 45%, the probability of concluding that it exceeds 45% (alpha) is 4.9%. If the one year DFS rate is really 75% the probability of concluding that it does not exceed 45% (beta) is 19%. Note that this is not a MiniMax or Optimum design as

described by Simon. Those designs would have provided for an unacceptably high probability of early stopping for futility with a reasonably good one year DFS rate such as 60%. One year DFS will be estimated using the method of Koyama and Chen (ref) which provides proper estimates and confidence intervals following two stage designs. Disease-free survival and overall survival over time will be estimated with Kaplan-Meier methods[34] with standard errors computed based on Greenwood's formula. Rates of relapse and acute and chronic graft-versus-host disease (all secondary objectives) will be estimated using the cumulative incidence methods described in Ruan and Gray.[35] Engraftment (secondary objective) will be summarized with the sample proportion of patients who engraft along with a corresponding exact 95% confidence interval, while regimen related toxicities (secondary objective) will be tabulated by category.

# 8 Adverse Event Reporting

Adverse events occurring following study registration, but prior to beginning transplant therapy will not be reported. Protocol related therapy does not begin until patients are admitted for their bone marrow transplant. Regimen related toxicity and adverse events will be monitored for and reported if required through day 100. Adverse Event and Toxicity assessments will be performed at each study visit per the study calendar. Following day 100, patients will still be followed for relapse and overall survival.

## 8.1 Definitions of Adverse Events with Commercially Available Agents

This trial utilizes commercially available agents for transplant therapy for patients with hematological malignancies. Commercial agents are those agents not provided under an IND but obtained instead from a commercial source. In some cases, an agent obtained commercially may be used for indications not included in the package label. In this case, the agent is still considered to be a commercial agent and the procedures described below should be followed.

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject whether or not it may have a causal relationship with this treatment. An AE includes significant exacerbation of any baseline medical condition including, but not limited to, the disease under study. Reporting requirements may include the following considerations: 1) the characteristics of the adverse event including the grade (severity); 2) the relationship to the study therapy (attribution); and 3) the prior experience (expectedness) of the adverse event.

Expected events are those that have been previously identified as resulting from administration of the agent. For commercially available agents, an adverse event is considered unexpected, for reporting purposes only, when either the type of event or the severity of the event is not listed in either of the following:

- The current NCI Agent-Specific Adverse Event List (provided in the Drug Information Section of this protocol).
- The drug package insert (for treatments with commercially available agents).

Except where otherwise specified, Common Terminology Criteria for Adverse Events (CTCAE) v.4 will be used to grade adverse events in this study.

### 8.2 Required Adverse Events Reporting

Therapy for ALL, with or without allo HCT, is associated with significant toxicity. These toxicities are generally viewed as an anticipated consequence of therapy rather than an adverse event. To summarize, adverse events with severity grades 1, 2, 3, and all expected grade 4 toxicities will not be reported to the IRB, as they are expected in patients undergoing allo HCT for ALL. Only unexpected grade 4 non-hematologic toxicity events with a possible, probable or definite relation to the study and all grade 5 events will be reported to IRB.

### 8.3 Hematologic toxicity and Definition of Primary Engraftment Failure

Failure to achieve a neutrophil count > 500/μL within 35 days of the stem cell infusion will be defined as primary engraftment failure and reported as an adverse event. If primary engraftment failure occurs, an action to obtain neutrophil recovery (such as the use of growth factors or stem cell boost) will be allowed. Neutrophil count recovery will continue to be monitored in these patients until they reach statistical endpoint.

### 8.4 Serious Adverse Event Reporting Procedures

All serious adverse events (SAE) which require reporting must be reported immediately (i.e. within 24 hours of awareness) to the Principal Investigator or designee, followed by written documentation to the IRB from the PI (including the PI's or designee's medical summary of the

SAE) within 7 days of the PI's knowledge of occurrence. A serious adverse event (SAE) is defined as**:** Any adverse event (experience) occurring that results in **ANY** of the following outcomes:

1) Death.
2) A life-threatening adverse drug experience.
3) Inpatient hospitalization or prolongation of existing hospitalization.
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

The research staff of Bone Marrow Transplant-Cell Therapy (BMT-CT) Program will coordinate the reporting process between the investigators and IRB as well as other applicable reporting agencies (FDA, CTEP, and NIH). Copies of all correspondence and reporting documents will be maintained in a regulatory file held by the research staff of BMT-CT Program.

# 9 Data Safety Monitoring

## 9.1 Data and Safety Monitoring Procedures

The Data and Safety Monitoring Board (DSMB) of The University of Alabama at Birmingham Comprehensive Cancer Center is the DSMB for this study. This committee is responsible for the quarterly review and monitoring the study's scientific progress, accrual rate and any serious adverse events.

In addition to the Cancer Center DSMB, BMT-CT Program will form a Data and Safety Monitoring Committee (DSMC) for the study. This committee will be composed of the PI, co-investigator(s), data manager or study coordinator and other members of the study staff involved

in the conduct of the trial. During the committee's quarterly meeting, the PI will discuss matters related to:

Enrollment rate relative to expectations, characteristics of participants

Safety of study participants (Serious Adverse Event & Adverse Event reporting)

Adherence to protocol (protocol deviations)

Completeness, validity and integrity of study data

Retention of study participants

These meetings are to be documented by data manager or study coordinator using the Protocol Specific Data and Safety Monitoring Report (DSMR), signed by the PI or co-investigator. The completed DSMR is to be sent to DSMB.

Similarly, protocol deviations are to be documented using the Notice of Protocol Deviation Form and requires the signatures of either data manager or study coordinator and the PI or co-investigator. These reports are to be sent to the IRB within 7 calendar days of awareness of the event and on a quarterly basis to the DSMB with the Protocol Specific DSMR.

### 9.2 Quality Assurance and Audits

The Quality Assurance Review Committee (QARC) of The University of Alabama at Birmingham Comprehensive Cancer Center performs quality assurance audits of investigator-initiated clinical trials. Audits provide assurance that trials are conducted in compliance with the protocol. Further, they ensure that study data are collected, documented and reported in compliance with Good Clinical Practices (GCP) Guidelines and regulatory requirements.

A QARC audit of each clinical trial is conducted annually. Audits occur within the month of the study's initial IRB approval (provided the trial is open, and study accrual is greater than five subjects).

All audit findings are reported by QARC to the DSMB. These findings are followed-up by the DSMB until they have been resolved.

The DSMB can also request QARC for a 'for cause' audit of the trial if the board identifies a need for a more rigorous evaluation of study-related issues.

A regulatory authority (e.g. FDA) may also wish to conduct an inspection of the study, during its conduct or even after its completion. If an inspection has been requested by a regulatory authority, the PI must immediately inform the IRB/DSMB that such a request has been made.

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## Appendix A: Performance Scores

| Karnofsky Performance Score |
|---|
| 100% – normal, no complaints, no signs of disease |
| 90% – capable of normal activity, few symptoms or signs of disease |
| 80% – normal activity with some difficulty, some symptoms or signs |
| 70% – caring for self, not capable of normal activity or work |
| 60% – requiring some help, can take care of most personal requirements |
| 50% – requires help often, requires frequent medical care |
| 40% – disabled, requires special care and help |
| 30% – severely disabled, hospital admission indicated but no risk of death |
| 20% – very ill, urgently requiring admission, requires supportive measures or treatment |
| 10% – moribund, rapidly progressive fatal disease processes |
| 0% – death. |

## Appendix B: Study Calendar

| Procedures | Screening Period Within 35 days of Study Registration / Day of Final Transplant Evaluation | Conditioning Phase | | | | | | | | Follow-Up Phase | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | -7 | -6 | -5 | -4 | -3 | -2 | -1 | Day 0 | +1 | +3 | +6 | +11 | Day 30 (±7) | Day 100 (±14) | Day 180 (±21) | Day 365 (±45) | 2 Years |
| Eligibility criteria, Informed Consent and medical/treatment history | X | | | | | | | | | | | | | | | | | |
| Pulmonary function test and Echocardiogram or MUGA | X | | | | | | | | | | | | | | | | | |
| HIV1 and HIV2 labs and Pregnancy Tests | X | | | | | | | | | | | | | | | | | |
| Liver Function Panels, Renal function and Hematology | X | | | | | | | | | | | | | | | | | |
| Physical exam , Vital Signs and Karnofsky Status[1] | X | | | | | | | | | | | | | X | X | X | X | X |
| Bone Marrow aspirate and biopsy[2], morphology and cytogenetics | X[2] | | | | | | | | | | | | | X[4] | X[4] | | X[4] | |
| Immune Reconstitution / Chimerism | | | | | | | | | | | | | | X | X | X | X | |
| Stem cell Infusion | | | | | | | | | X[3] | | | | | | | | | |
| GVHD Prophylaxis- Tacrolimus[5] | | | | | | X | | | | | | | | | X | | | |
| GVHD Prophylaxis- Methotrexate[6] | | | | | | | | | | X | X | X | X | | | | | |
| GVHD Prophylaxis – rATG[11] | | X | X | X | | | | | | | | | | | | | | |
| Fludarabine[9] | | X | X | X | X | | | | | | | | | | | | | |
| Total Body Irradiation[10] | | | | | | X | X | X | | | | | | | | | | |
| Acute GVHD assessment[7] | | | | | | | | | X | | | | | X | X | | | |
| Chronic GVHD assessment[8] | | | | | | | | | X | | | | | | X | X | X | X |
| Adverse Events and Toxicity Monitoring | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |

1. Karnofsky Performance Score will be assessed within 14 days prior to registration.
2. A bone marrow aspirate and biopsy will be performed within 35 days of registration to assess morphology and cytogenetics.
3. If stem cell infusion requires more than one day, then the first day after the last stem cell infusion will be defined as day 1.
4. A bone marrow aspirate will be collected whenever relapse is suspected. Patients that relapse will be followed for survival.
5. Tacrolimus (0.03mg/kg/day) will be given starting day -3. Patients will convert to oral tacrolimus when tolerated. Tacrolimus will be continued till day +100 and tapered if there are no signs of active GVHD.
6. Methotrexate 5mg/$m^2$ will be given on day 1, 3, 6 and 11. If sever mucositis develops, Leucovorin 5mg/$m^2$ on day 4,7,and 12 if severe mucositis develops
7. Acute GVHD will be assessed weekly (±3 days) till 100 days post-transplant.
8. Chronic GVHD will be assessed monthly (±14 days) till 1 year post transplant.
9. Fludarabine 40 mg/$m^2$/day
10. Total Body Irradiation 200cGy/fraction x 2 on days -3.-2 and -1.
11. rATG (1.5mg/kg on Day -7, -6, and -5) given for MUD patients only.